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REPORT: LABORATORY VALIDATION OF FIELD EXPERIMENTS CARRIED OUT AT THE UPPER OTTAWA STREET LANDFILL, AUGUST 1982

July 1983

INTRODUCTION

In August 1982 an attempt was made to measure the mutagenicity of gases emanating from vents on the Upper Ottawa Street Landfill Site. To this end 3 methods, modifications of the Ames assay, were designed and carried out (Report, Sept. 1982). None of these methods gave an indication of the presence of mutagenic compounds in the gases tested. None of the methods had, however, been validated under controlled conditions in the laboratory, leaving the significance of this result in doubt. Below is described an attempt to do this. The experimental methods used in the field have been repeated in the laboratory using the known mutagen ethylene dibromide.

MATERIALS AND METHODS

The materials and methods used closely resembled those described in the September 1982 Report. Only strains TA98 and TA100 were used. The characteristics of these strains (Histidine requirement, rfa character, ampicillin resistance and uvrB deletion) were confirmed at the time of the experiments.

"Bag" method

Ampicillin (0.1ml of an 8mg/ml solution in 0.02N NaOH) was included in the top agar of each plate. Ethylene dibromide vapour was generated by pumping compressed air onto the chemical in a small side-arm flask. During the first experiment the chemical was at room temperature and during the second, on ice. The exhaust from the flask was forced into the "bag" through loosely packed cotton dressing as described (September 1982, Report).

The method was carried out on two occasions, details of which are given below.

	Assay I	Assay II
Time on ice before exposure	1 hr. 20 min.	2 hr.
Flow rate into bag (approx.)	2.5 1/min.	5 1/min.
Weight loss of chemical	N.D.*	14.66 g
Total duration of exposure	230 min	120 min.
Estimated abundance of chemical in air	N.D.*	11.3 ppm
Time on ice after expo-	1 hr. 45 min.	2hr. 20 min.

* chemical was accidently spilt before second weighing

"Desiccator" method

Plates were poured and arranged in desiccators, inverted, without lids and stacked on aluminum spacers. The desiccators were then placed in a cold room; for 1 hr. 30 min. on the first occasion that the assay was carried out and for 4 hr. 20 min. on the second. Ethylene dibromide was introduced to the plates after partial evacuation (Approx. 70%) of each desiccator by allowing air to re-enter through glass vacutainers containing a known volume of the chemical which rapidly evaporated. When evaporation was seen to be complete and atmospheric pressure was restored, the desiccators were sealed. They were then returned to the cold room for 1 hr. 15 min. (first assay) or 1 hr. 50 min. (second assay) before incubation at 37°C.

"Bubbling" method

Overnight cultures (8 ml) of TA98 and TA100 which had been on ice for approx. 2 hr. were placed in a water bath at 37°C. Air was pumped at a rate of 4 cc/min. using a peristaltic pump over a known weight of ethylene dibromide held in vacutainers in the same water bath. The exhaust was bubbled through each culture. Samples (approx. 0.5 ml) were taken at intervals for 3 hr. and placed in tubes in the water bath. Unexposed controls were left at 37°C for the duration of the experiment. At the end of exposure the tubes were placed on ice for approx. 1 hr. and the remaining ethylene dibromide weighed. Cells were then plated according to the normal Ames

procedures except that the top agar contained ampicillin, prepared as described above.

	TA98	TA100
Flow rate (approx.)	4 cc/min.	4 cc/min.
Duration of experiment	180 min.	180 min.
Total weight loss of chemical	42.6 mg	38.3 mg
Estimated abundance of ethylene dibromide in air	27 ppm	24 ppm

RESULTS

In all assays a positive response was observed with both TA98 and TA100. In agreement with already published data TA100 was more sensitive than TA98 to ethylene dibromide. Results for the 'bag' method are shown in Tables I and II, for the 'desiccator' method in Tables III and IV and for the bubbling method in Table V. The desiccator method appeared to be the most sensitive of the 3; extrapolation of results indicated that as little as 55 ppb ethylene dibromide could be detected by TA100.

The 'bag' assay appeared to be less sensitive in the second experiment (Table II). However, in spite of a more rapid rate of air flow into the bag, the chemical was held on ice on this occasion - the evaporation rate of ethylene dibromide in the first experiment could not be calculated.

It was clear that all assays were able to readily detect the mutagenic action of ethylene dibromide. The procedures used on the landfill in August 1982 were closely followed in these experiments and it is likely that were mutagenic compounds present in sufficiently high concentrations they would have been detected at that time.

Although the desiccator assay appeared to be the most sensitive method, plates were exposed to the drug for longer times in this method than in the others. Both this and the bubbling method suffer when complex and varying mixtures of compounds are being evaluated (the situation on the landfill), because only a limited volume of gas can be sampled.

TABLE 1 - "BAG" ASSAY - 1

Treatment	TA 98		TA 100	
	MEAN	SEM	MEAN	SEM
Control	11.0	0.9	120.8	9.4
20 min.	45.3	14.9	823.0	193.0
40 min.	40.3	8.6	718.7	99.6
60 min.	49.0	9.0	1045.0	49.0
110 min.	72.3	8.1	1608.0	64.0
140 min.	46.3	5.7	1134.7	44.6
170 min.	74.3	9.1	1458.7	170.2
200 min.	71.3	4.3	1629.3	134.1
230 min.	51.0	2.3	1280.0	59.2
4-NQO	+ve*		+ve*	

* Clear positive result obtained after spot test.

Experiment 090783   Scorer DB.

TABLE II - "BAG" ASSAY - 2

Treatment	TA 98		TA 100	
	MEAN	SEM	MEAN	SEM
Control	15.3	2.0	194.3	27.3
15 min.	12.0	2.3	305.3	12.0
30 min.	19.7	2.3	378.7	39.3
45 min.	12.0	1.7	427.3	39.0
60 min.	26.3	3.7	576.3	34.3
75 min.	31.0	0.0	680.7	86.1
90 min.	41.0	1.0	717.7	50.6
105 min.	47.7	11.0	740.0	73.5
120 min.	65.0	4.6	980.0	96.3
4-NQO	+ve*		+ve*	

* Clear positive result obtained after spot test

Experiment 120783 Scorer DB.

TABLE III - DESICCATOR ASSAY - 1

ETHYLENE DIBROMIDE µl/DESICCATOR	PPM	TA 98		TA 100	
		MEAN	SEM	MEAN	SEM
0	0	29.0	2.9	295.5	7.5
1	0.11	46.7	2.6	748.3	15.3
10	1.10	128.7	10.2	2901.3	531.6
67	11.10	294.0	5.2	TNTC	
4-NQO		+ve *		+ve *	

* Clear positive result obtained after spot test.

TNTC Too numerous to count

Experiment 070783 Scorer DB.

TABLE IV - DESICCATOR ASSAY - 2

ETHYLENE DIBROMIDE μl/DESICCATOR	PPM	TA 98		TA 100	
		MEAN	SEM	MEAN	SEM
0	0	14.7	1.5	186.7	9.0
0.67	0.11	23.3	1.9	576.0	49.0
2.5	0.28	35.3	2.7	770.0	88.5
5.0	0.55	41.7	2.7	969.3	53.2
4-NQO		+ve *		+ve *	

*Clear positive result obtained after spot test.

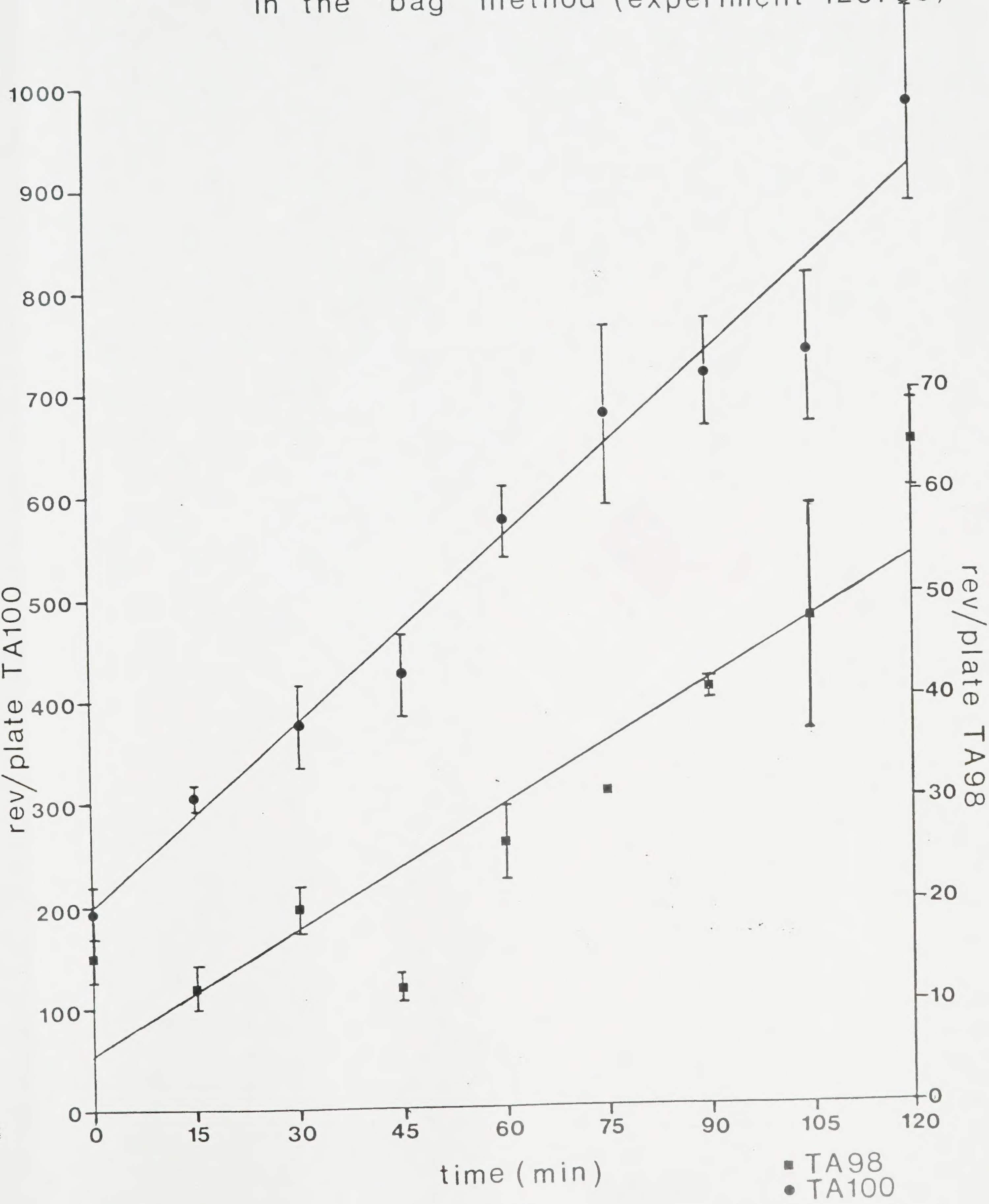
Experiment 120783 Scorer RM.

TABLE V - "BUBBLING" ASSAY

Treatment	TA 98		TA 100	
	MEAN	SEM	MEAN	SEM
Control	16.0	4.2	187.7	20.2
15 min.	44.5	12.5	417.0	43.3
30 min.	91.0	8.7	1422.0	206.6
45 min.	83.0	9.1	1260.0	78.2
60 min.	123.0	11.7	1341.3	76.7
90 min.	136.3	9.0	1733.3	90.1
120 min.	147.0	18.0	2628.0	52.0
150 min.	171.0	8.5	2344.0	238.7
180 min.	167.0	16.7	2744.0	188.1
4-NQO (0.5 μg/plate)	509.5	29.5	>1000	

Experiment 090783 Scorer DB.

Fig 1 - Mutagenicity of ethylene dibromide in the 'bag' method (experiment 120783)



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